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Drought Resistant Impacts on Vital and Biochemical Traits of Micropropagated *Khaya senegalensis* (Desr.) A. Juss. Plant

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Abstract

This investigation was conducted to develop a protocol for shoot proliferation in *Khaya senegalensis* (Desr.) A. Juss., as a multipurpose tree with commercial and environmental values. MS culture medium with or without 2gl^{-1} activated charcoal (AC) and benzylladenine (BA) (0.0, 0.5 and 1.0 mgl^{-1}) individually or combined with indol-3-butyric (IBA) (0.0, 0.05, 0.1 and 0.2 mgl^{-1}) was used. Polyethylene glycol (PEG) was added at various levels (0.0, 0.5, 1.0, 1.5, and 2.0%) to the optimization culture medium and melatonin at 0, 1, and 2 mgl^{-1}. Using MS medium supplemented with 1.0 mgl^{-1} of BA + 0.05 mgl^{-1} of IBA resulted in the most proliferated shoots with the highest rooting percentage at the presence of 2 gl^{-1} of AC. The enhancement effect of melatonin at 2 mg/l was noticed on proliferated shoots under 0.5 and 1% of PEG. Melatonin treatments resulted in a significant increase in photosynthetic pigment contents ($P \leq 0.05$). The highest concentrations of PEG (2%) and melatonin (2 mgl^{-1}) individually or interacted were more effective for increasing secondary metabolites such as total phenol, tannin, flavonoid, and antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD). This study could be useful in mitigating drought stress using melatonin as a regulator for physiological and biochemical processes.

Keywords; African mahogany, PEG, drought mitigation, secondary metabolites, and antioxidant enzymes

1. Introduction

Khaya senegalensis (Desr.) A. Juss., known as African mahogany, belongs to the Meliaceae family which is native to Africa, It grows well in Egypt as a shade and avenue tree [1],[2] it is a commonly used tree in several plantations existing in Egypt such as Serapium plantation Ismailia Governorate, where its volume reached 102.5 m^{3}ha^{-1} [3] Additionally, Egyptian Chinese friendship woody forest Sadat City, Minufiyah Governorate, Egypt Egyptian-Japanese woody forest at Wadi Al-Natron, Behaira Governorate [4], also it can grow in the western Delta region and reclaimed lands, due to the ability to adapt environmental conditions as well as its economic and aesthetic importance of *K.senegalensis*, so, it is included in afforestation programs in Egypt [5]. Recently, it has been implicated in new studies assessing the biodiversity and conservation of woody species at Giza Zoo, Egypt, as a botanical garden that allows investigators to innovate modern techniques to conserve plant diversity, to achieve Egyptian sustainable development by 2030 [6]. *K. senegalensis* is a hardwood ornamental tree, it can grow up to 35 m in height and 1.5 m in diameter on fertile soil. It has numerous medicinal uses antimicrobial, and anthelmintic, also it is also used in the treatment of a wide variety of infections fungal and protozoal, as well as in the treatment of cancers [7]. In addition to its uses in furniture making, construction, and decorative veneers. Also, its seeds contain high oil levels, so it has the potential as a biofuel source with excellent commercial value which can be used for cosmetic and anther multi purposes [8].

Drought and its negative impacts on plants' growth and productivity will become a considerable threat in the future, where drought-stress induced physiological changes in plants [9], which sparked great interest among researchers to identify the

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effective mechanisms of plant adaptation under water deficit.

By creating conditions that are similar to those established when plants are exposed to dryness in the field environment, drought conditions can be applied *in vitro*. Under deficit water conditions, plants accumulate solutes and osmolytes in plant cells. Because of this, large molecular weight solutes like polyethylene glycol (PEG), sucrose, sorbitol, mannitol, and sorbitol are good candidates to impose physiological drought under *in vitro* circumstances [10]. These osmolytes maintain the structural integrity of proteins and cell membranes during conditions of dehydration stress [11].

Melatonin (N-acetyl-5-methoxy-tryptamine) was identified and quantified in plants in 1995, as an indoleamine neurohormone [12] and [13], it was classified as a newly discovered plant growth regulator, so melatonin has since been specified as playing vital roles in responses of growth, development, and stress in several plants, it can stimulate the expression of gene leading to the antioxidant enzymatic and non-enzymatic system's activation to protect the plant cell of the negative impact of different abiotic stresses [14]. Moreover, melatonin might increase the mitochondrial efficiency of the electron transport chain, which boosts the stability of biofilms by protecting them from oxidative damage [15], the *in vitro* culture system represented a unique scope compared to field trials and greenhouse, where all cultures are maintained in aseptic conditions, wherever could examine the roles of melatonin in plants, which are helping to solidify the role of melatonin in plant processes [16].

Therefore, the present study aimed to establish an *in vitro* protocol for shootlet proliferation for massive production of *K.senegalensis*, as well as evaluate its response to additive treatments of melatonin under drought stress conditions.

2. Materials and Methods

2.1. Chemicals and reagents

Folin-Ciocalteau reagents, Gallic acid, Quercetin, Pyrogallol, DTNB, Sodium azide, and Aluminum chloride were obtained from (Sigma Chemical Co **,.** St.Louis, MO, USA).

2.2. Plant material

The present investigation was conducted at the Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, Central Laboratories, National Research Centre (NRC), Egypt, during the years 2021 and 2022. *K. senegalensis* seeds were harvested from the trees maintained at the National Research Centre farm for research and production in Al-Emam Malek village, Al-Nubaria region, Al-Behira Governorate, Egypt.

2.3. Procedure layout

2.3.1. Surface sterilization

Healthy uniform un-coated seeds of *K. senegalensis* were surface sterilized under aseptic conditions in a laminar air flow hood. Seeds were disinfested by rinsing for 30 sec in 70% (v/v) ethanol followed by 15% commercial sodium hypochlorite solution then washed three times with sterile autoclaved distilled water. The sterilized seeds were left to germinate on basal medium MS supplemented with 2.5% (w/v) sucrose and solidified with agar at 0.7% (w/v) which was adjusted to 5.7 ± 0.2 pH medium, followed by incubation in the dark until germination.

2.3.2. Culture medium and incubation condition

Initially, to establish an *in vitro* protocol for shootlet proliferation, four weeks old germinated seeds, the epicotyls were excised and transferred to MS modified with De Fossard medium vitamins [17] (4.9 mgl^{-1} Nicotic acid+0.88 mgl^{-1} Calcium pantothenate+3.6 mgl^{1} ribophlavin+ 1.8 mg l^{-1} Ascorbic acid+1.4 mg l^{-1} choline chloride+14.5 mg l^{-1} L-cystain) and supplemented with 25 g l^{-1} sucrose, 7g l^{-1} agar. For optimize the suitable proliferation medium, three benzyladenine (BA) concentrations (0.0, 0.5, or 0.1 mg l^{-1}) individually or in combination with indol-3-butyric acid (IBA) (0.0, 0.05, 0.1 and 0.2 mg l^{-1}) at the presence or absence of activated charcoal (AC) at 2g l^{-1}.

On a growth chamber, cultures were incubated at 25±2EC under photoperiod 16h of fluorescent light with 30µmol $m^{2}sec^{-1}$.

Survival of explants (%), shootlets number/explant, shootlets length (mm), leaves number /shootlet as well as rooting parameters (rooting (%), roots number/shootlets and root length (mm)) were recorded after three months.

2.4. Micropropagation ability under drought stress

The optimized culture medium (1 mgl^{-1} of BA + 0.05 mgl^{-1} of IBA at presence of $2gl^{-1}$ activated charcoal) for *in vitro* shooting and rooting abilities

which were selected from the previously tested multiplication media included polyethylene glycol (PEG) (water stress agent) at five levels (0.0, 0.5, 1.0, and 2.0 %) as well as melatonin at 0.0, 1.0 and 2.0 mgl^{-1} to alleviate drought stress.

In vitro proliferated shootlet criteria were represented in the survived explants (%), shootlets number/explant, shootlets length (mm), leaves number/shootlet and the *in vitro* root growth parameters (rooting percentage %, roots number/shootlets, and root length mm), data were recorded after three months.

2.5. Biochemical analysis

Photosynthetic pigments: chlorophyll a, b and total carotenoids were determinate according to Saric et al. [18]

Shootlets extraction: Shootlets of *K. senegalensis* (10g) were soaked in 100 ml of ethanol 80 % and were shacked for 48h at room temperature. The extracts were filtered and extracted twice.

Phenolic compounds: The final extract was used for the assay of the phenolic compound. Total phenols were assayed using Folin–Ciocalteu's reagent, according to Singleton and Rossi [19]. The tannin content was determined according to Tambe et al. [20]. Total flavonoid was assayed by the method of Zhishen et al. [21].

Antioxidant enzymes extraction and determination of enzyme activities: *K. senegalensis* fresh shootlets (0.1g) were ground and then extracted with ice-cold (5 ml) buffer phosphate at PH 7.4. The supernatant was collected after the homogenate was centrifuged for 30 min at 10,000 rpm. The resulting supernatant was used for the determination of enzyme activities.

The catalase activity was determined by the method of Nakano and Asada [22].

The enzyme Superoxide dismutase activity (SOD) was measured according to Marklund and Marklund [23].

Statistical analysis: The treatments' means were compared for significance by Duncan's New Multiple Range test [24] at a 5% level of probability using MSTAT Computer Program (MSTAT Development Team) [25]. For the multiplication optimization stage, the experiment was designed as one factor in a completely randomized block design. For micropropagation ability, chemical changes and enzyme activity under drought stress, analysis of variance was estimated as two factorials in a completely randomized block design. The data were statistically analyzed according to Steel and Torrie [26].

3. Results and Discussion

3.1. Culture medium optimizing

A protocol for shoot proliferation was developed in order to promote the *in vitro* growth of *Khaya senegalensis*, a crucial species for forestry and medicinal purposes. As shown in Table1, nodal explants were introduced on MS culture medium with or without $2gl^{-1}$ of activated charcoal (AC) and supplemented with BA (0.0, 0.5 and 1.0 mgl^{-1}) individually or combined with IBA (0.0, 0.05, 0.1 and 0.2 mgl^{-1}). It was noticed that all cultured explants were able to survive at 100% on all examined treatments. On the other hand, the results were recorded for rooting ability on all used treatments at the absence of charcoal in the culture medium led to non-rooted plants (Table 1). Meanwhile, using BA at 1.0 mg/l caused the highest numbers of both shoots/explant and leaves formed/shootlet (7.67 and 21.33, respectively). Adding IBA (0.05 to 0.2 mgl^{-1}) to the culture medium containing BA caused a decrease in the ability of shootlet proliferation gradually to attain the lowest value (4.0). However, the most elongated shoots (86.68 mm) were obtained on MS culture medium free of any hormones (Table 1).

Numerous studies have shown that cytokinin, particularly BA, can promote the formation of axillary buds but, it restricts shoot elongation especially at high concentrations [27]. Similar results were found by Hung and Trueman [28] on *Khaya senegalinsis*, who observed that the length of shoots was unaffected by the addition of BA to the induction media.

From the results in Table (1), using activated charcoal at $2gl^{-1}$ in the culture medium containing 0.5 mgl^{-1} of BA led to the longest shoots (135 mm) with the highest number of leaves (10.67). For root formation, supplementation of AC to all tested culture media containing BA combined with IBA at all tested concentrations caused high rooting percentage (100%).

Using BA at 0.5 mgl^{-1} with 0.2 gl^{-1} of IBA in the culture medium resulted in the longest roots (125.40 mm). While 1.0 mgl^{-1} of BA + 0.05 mgl^{-1} of IBA + 2 gl^{-1} of activated charcoal caused more proliferated shoots with the highest rooting percentage (100%)

and root number (2.67) that was not significantly different from the highest one.

Table 1. Shooting and rooting abilities of *in vitro* grown *Khaya senegalensis* under various levels of BA and IBA (mg l^{-1}) without and/or with activated charcoal (2g l^{-1})

Treatments	Survival %	Number of shootlets/ Explant	Length of shootlets (mm)	Number of leaves /shootlet	Rooting %	Number of roots /shootlet	Length of roots (mm)
Without activated charcoal (2g l^{-1})							
MS free of hormones	100A	1.00 E	86.68 A	3.00 F	0	0	0
MS + 0.5 mg L^{-1} BA	100A	4.78 CD	72.00 D	19.00 AB	0	0	0
MS + 1 mg L^{-1} BA	100A	7.67 A	75.00 B	21.33 A	0	0	0
MS + 0.5 mg L^{-1} BA + 0.05 mg L^{-1} IBA	100A	7.00 AB	72.06 D	17.00 BC	0	0	0
MS + 0.5 mg L^{-1} BA + 0. 1 mg L^{-1} IBA	100A	6.33 ABC	73.74BCD	8.67 E	0	0	0
MS + 0.5 mg L^{-1} BA + 0. 2 mg L^{-1} IBA	100A	4.00 D	66.75 F	12.67 D	0	0	0
MS + 1 mg L^{-1} BA + 0.05 mg L^{-1} IBA	100A	5.33 BCD	74.68 BC	14.67 CD	0	0	0
MS + 1 mg L^{-1} BA + 0. 1 mg L^{-1} IBA	100A	5.89 ABCD	73.09 CD	13.33 CD	0	0	0
MS + 1 mg L^{-1} BA + 0. 2 mg L^{-1} IBA	100A	4.67 CD	69.78 E	11.00 DE	0	0	0
With activated charcoal (2g l^{-1})							
MS free of hormones	100 A	1.00 C	100.00 C	5.00 BC	33.33 D	0.34 E	38.33 H
MS + 0.5 mg L^{-1} BA	100 A	1.33 BC	135.00 A	10.67 A	66.67 B	1.67 CDE	45.00 G
MS + 1 mg L^{-1} BA	100 A	1.67 BC	95.00 D	5.67 BC	50.00 C	1.00 DE	40.00 H
MS + 0.5 mg L^{-1} BA + 0.05 mg L^{-1} IBA	100 A	2.33 AB	80.00 F	2.67 DE	100 A	2.00 BCD	85.00 E
MS + 0.5 mg L^{-1} BA + 0. 1 mg L^{-1} IBA	100 A	1.33 BC	75.00 G	2.33 E	100 A	3.33 AB	103.00 B
MS + 0.5 mg L^{-1} BA + 0. 2 mg L^{-1} IBA	100 A	1.33 BC	73.35 G	1.67 E	100 A	3.33 AB	125.40 A
MS + 1 mg L^{-1} BA + 0.05 mg L^{-1} IBA	100 A	3.00 A	86.45 E	6.67 B	100 A	2.67 ABC	78.26 F
MS + 1 mg L^{-1} BA + 0. 1 mg L^{-1} IBA	100 A	2.00 ABC	103.33 B	4.67 BCD	100 A	3.67 A	100.00 C
MS + 1 mg L^{-1} BA + 0. 2 mg L^{-1} IBA	100 A	1.78 BC	81.25 F	3.67 CDE	100 A	1.00 DE	95.00 D

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. Ms: Murashige and Skoog medium, BA: Benzyladenine, IBA: Indol-3-butyric acid.

This means that the above-mentioned medium (MS+ 1 mg^{-1} of BA + 0.05 mgl^{-1} of IBA + 2 gl^{-1} of activated charcoal) was the optimized one to obtain the highest shootlets multiplication with high rooting ability as shown in Fig.1 (a, b). These findings are consistent with the findings of other researchers who studied the varying effects of cytokinin and auxin on the shoot regeneration of many woody species.

The clear response of shootlet multiplication to BA additive to the culture medium suggested that BA is thought to be the cytokinin that promotes cell division and overcomes apical dominance [29]. Besides their role in the plant cell's biosynthesis of RNA, enzymes, and proteins, which also induces bud growth [30]. The use of cytokinins and auxins in the multiplication stage is necessary, and the role of cytokinins at this stage is crucial to breaking apical dominance in buds and inducing subsidiary meristems that grow into shoots [31].

The positive effect of charcoal on both shooting and rooting abilities is observed in the present study, especially with cytokinin and auxin combination in the culture medium. The effect of IBA on root formation due to auxins' beneficial effects on promoting root production results from their primary physiological action, which is the stimulation of adventitious roots [32].

3.2. Micropropagation ability under drought stress

Comparing *in vitro* applications to field research for drought screening, *in vitro* applications can be a smart and simple approach. In the present work, the in vitro cultured explants on optimized multiplication medium (MS+ 1 mgl^{-1} of BA + 0.05 mgl^{-1} of IBA + 2gl^{-1} activated charcoal) were subjected to variant PEG concentrations (0.0, 0.5, 1.0, 1.5 and 2%) that represent five drought stress levels as well as melatonin at 0, 1 and 2 mgl^{-1} to alleviate drought stress (Table 2). The explants could survive (100%) under all treatments. However, there was a clear effect of PEG concentration increasing to 2% in the medium on *in vitro* cultured explants that caused depression in all of the shooting and rooting recorded characters (Fig.1:c). While using melatonin in the multiplication medium showed ascending increments for all recorded characters with increasing the concentration of melatonin from 0 to 2 mgl^{-1} (Fig.:d). The interaction effect showed enhancement of melatonin at 2 mgl^{-1} on proliferated shoots formed per explants under 0.5, and 1% PEG which recorded

2.50, and 1.79, respectively, as compared to those cultured on drought stress without melatonin that recorded 1.78 and 1.23, respectively. The proliferated shoots under stress above 1% PEG showed non-significant differences due to using melatonin. It could be noticed that the shoot elongation, number of leaves, and all rooting characters (rooting% and number of root) showed an increase under all examined drought stress (0.5-2.0%) when melatonin at 2 mgl^{-1} was added to the culture medium (Table 2).

Table 2. Micropropagation ability of *Khaya senegalensis* at different concentrations of melatonin under drought stress.

Treatment A	Treatment B	Survival %	Number of shootlets/ explant	Length of shootlets (mm)	Number of leaves /shootlet	Rooting %	Number of roots /shootlet	Length of roots (mm)
0% PEG	0 mg L^{-1} Melatonin	100 A	2.74 C	82.50 FG	5.67F	100.00A	2.00D	93.32C
	1mg L^{-1} Melatonin	100A	3.53 B	100.00 B	12.00B	100.00A	2.50D	100.00B
	2 mg L^{-1} Melatonin	100A	5.37 A	113.55 A	22.53A	100.00A	3.51A	112.50A
0.5% PEG	0 mg L^{-1} Melatonin	100A	1.78 EF	88.00E	7.33E	55.51E	1.00F	35.00J
	1mg L^{-1} Melatonin	100A	2.00 DE	89.44D	8.10D	66.75C	2.43C	61.67E
	2 mg L^{-1} Melatonin	100A	2.50 CD	94.27C	9.02C	85.15B	3.00B	64.81D
1% PEG	0 mg L^{-1} Melatonin	100A	1.23 FG	81.45G	4.25IJK	47.71G	1.00F	36.00J
	1mg L^{-1} Melatonin	100A	1.50 EFG	90.00D	4.44HIJK	53.33F	1.32E	49.77F
	2 mg L^{-1} Melatonin	100A	1.79 EF	83.47F	5.23FG	60.00D	2.00D	45.00G
1.5% PEG	0 mg L^{-1} Melatonin	100A	1.00 G	78.33I	4.11JK	33.30L	1.00F	35.00J
	1mg L^{-1} Melatonin	100A	1.33 EFG	80.08H	4.28IJK	42.00J	1.00F	40.00H
	2 mg L^{-1} Melatonin	100A	1.67 EFG	81.85G	4.84GHI	46.11H	1.95D	39.56HI
2% PEG	0 mg L^{-1} Melatonin	100A	1.00G	76.11J	4.00 K	22.22M	1.00F	35.00J
	1mg L^{-1} Melatonin	100A	1.22FG	78.33I	5.00 GH	36.00K	1.30E	35.00J
	2 mg L^{-1} Melatonin	100A	1.50EFG	81.67G	4.78 GHIJ	44.00I	2.00D	38.43I
Mean	0% PEG	100A	3.88A	98.69A	13.40 A	100.00A	2.67A	101.90A
	0.5% PEG	100A	2.09B	90.57B	8.15B	69.14B	2.14B	53.83B
	1% PEG	100A	1.50C	84.98C	4.64C	53.68C	1.44C	38.59C
	1.5% PEG	100A	1.33C	80.09D	4.41C	40.47D	1.32C	38.19D
	2% PEG	100A	1.24C	78.70E	4.60C	34.07E	1.43C	36.14E
Mean	0 mg L^{-1} Melatonin	100A	1.55C	81.28C	5.07C	51.75C	1.20C	46.86C
	1mg L^{-1} Melatonin	100A	1.92B	87.57B	6.77B	59.62B	1.71B	57.29B
	2 mg L^{-1} Melatonin	100A	2.57A	90.96A	9.28A	67.05A	2.49 A	60.06 A

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. Ms: Murashige and Skoog medium, BA: Benzyladenine, IBA: Indol-3-butyric acid.

The inhibition effect of PEG on the shooting and rooting abilities of micropropagated plants was confirmed by Sehgal et al. [33] that morphological, physiological, and biochemical criteria are influenced by drought stress as a result of the activation of stress-responsive factors. PEG solutions mimic dry media compared to compounds that have low molecular weight, PEG is not involved in the metabolism of plants, and it can penetrate the membrane of cells with solute. However, it causes water stress by reducing the water potential of nutrient solutions [34] and [35]. The positive effect of melatonin on *in vitro-grown* explants under drought stress was agreed with Murch and Saxena [36] who noticed that melatonin controls important plant processes like morphogenesis and rhizogenesis. Additionally, melatonin encourages plant development by increasing the effectiveness of carbon absorption [37]. Moreover, melatonin maintains cell turgor, which increases the capacity of stomatal opening and conductivity [38]. This improved CO_2 and water transport from the stomata

to the leaves eventually favours photosynthesis in melatonin-treated plants [39]. The *in vitro* survived and rooted *K. senegalensis* plantlets were successfully adapted in peat moss and sand (1: 1 v/v). The successfully acclimatized plants were 85%, as shown in Fig.1 (e).

Fig. 1 (a-e): *In vitro* shooting and rooting ability of *Khaya senegalensis* (Desr.) A. Juss., (a) Shootlets development that were produced from using MS+ 1 mg^{-1} of BA + 0.05 mgl^{-1} of IBA + 2 gl^{-1} of activated charcoal (optimized culture medium), (b) Rooting of shootlets cultured on the optimized culture medium, (c) Shootlets cultured on the optimized culture medium + PEG at 2%, (d) Shootlets cultured on the optimized culture medium + melatonin at 2mgl^{-1}, and (e) Acclimatized *K. senegalensis* plants.

3.3. Photosynthetic pigments content

Concerning pigments content in obtained shootlets from various drought levels, a similar trend of shooting and rooting abilities under drought stress was observed (Table 3). The lowest Chl.a, b and carotenoids contents (29.90, 13.69 and 25.53 mg/100 g F.W., respectively) were found with the highest concentration of PEG (2%) as compared to the control that recorded the highest values (68.36, 25.15 and 53.15 mg/100g F.W., respectively). While using melatonin in the culture medium showed a clear stimulation effect on shootlets pigments contents. This positive effect of melatonin was increased when interacted with PEG and led to a parallel increase in pigments content. From the recorded results, it was noted that the formation of pigments was improved in the growing shoots under drought stress, even at high levels, when melatonin was used as compared to those formed under all drought stress levels only.

Drought stress causes oxidative stress that damages plant cells by disrupting redox balance in the cells, which leads to stimulation of reactive oxygen species (ROS), leading to decreased formation of photosynthetic [40]. Earlier researchers suggested that water stress limits surrounding CO_2 distribution to the carboxylation sites, which is occured by stomatal closure, the main factor which reduces photosynthetic rate [41]. The role of melatonin in the enhancement of total chlorophyll content in *Solanum lycopersicum* L. and *Lupinus termis* L. under the conditions of water stress was also indicated [42] [43]. Supported investigations pointed out that reduced chlorophyll decomposition and down-regulation of genes that promote senescence are also associated with melatonin-mediated enhancements in photosynthesis [44]. Melatonin enhances photosynthesis, transpiration, and stomatal conductance while preventing the breakdown of the chlorophyll molecule during drought stress [45]. Under drought stress, melatonin also increases the amount of photosynthetic accessory pigments like carotenoids [46].

Table 3. Photosynthetic pigments content (mg/100 g F.W.) of *in vitro Khaya senegalensis* shootlets grown at different concentrations of melatonin under drought stress

Treatment A	Treatment B	Chlo- a	Chlo-b	Total caroten.
0% PEG	0 mg L^{-1} Melatonin	60.76C	24.04C	54.51A
	1mg L-1 Melatonin	67.61B	24.66B	50.09B
	2 mg L-1 Melatonin	76.71A	26.74A	54.85A
0.5% PEG	0 mg L-1 Melatonin	41.47G	19.85D	44.74C
	1mg L-1 Melatonin	45.44F	18.89E	40.75E
	2 mg L-1 Melatonin	54.02D	19.12E	43.34D
1% PEG	0 mg L-1 Melatonin	27.78J	13.94H	24.55I
	1mg L-1 Melatonin	41.24G	15.82G	33.47F
	2 mg L-1 Melatonin	46.18E	15.76G	31.56G
1.5% PEG	0 mg L-1 Melatonin	23.97K	12.46J	19.50L
	1mg L-1 Melatonin	30.89I	16.36F	29.05H
	2 mg L-1 Melatonin	41.27G	14.06H	24.36I
2% PEG	0 mg L-1 Melatonin	20.15L	13.01I	21.61K
	1mg L-1 Melatonin	30.30I	12.74IJ	23.20J
	2 mg L-1 Melatonin	39.25H	15.33G	31.79G
Mean	0% PEG	68.36A	25.15A	53.15A
	0.5% PEG	46.98B	19.29B	42.94B
	1% PEG	38.40C	15.17C	29.86C
	1.5% PEG	32.04D	14.29D	24.30E
	2% PEG	29.90E	13.69E	25.53D
Mean	0 mg L-1 Melatonin	34.83C	16.66C	32.98C
	1mg L-1 Melatonin	43.10B	17.69B	35.31B
	2 mg L-1 Melatonin	51.48A	18.20A	37.18A

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. PEG: Polyethylene glycol.

3.4. Secondary metabolites (total phenols, tannins, and flavonoids)

As indicated in Table (4), the *in vitro* cultures grown with high PEG concentration (2%) showed significant induction and resulted in the highest significant value (29.80, 14.17, and 7.82 µg/g, respectively) of all estimated secondary metabolites (total phenols, tannins, and flavonoids) as compared with untreated cultures with PEG which recorded the lowest value (17.53, 6.62, and 4.27 µg/g, respectively). Furthermore, the positive effect of melatonin treatment was noticed on these compounds whereas, using melatonin at $2gl^{-1}$ showed the highest contents (24.54, 11.63, and 6.39 µg/g). The interaction effect of PEG and melatonin showed that the obtained shootlets from the culture medium supplemented with high concentrations of PEG (2%) and melatonin ($2gl^{-1}$) contained the highest secondary metabolites (32.13, 16.90, and 9.70 µg/g). Introducing melatonin in the culture medium that contained PEG (0-2%) caused a clear enhancing effect on total phenols, tannins, and flavonoids. The increase of phenolic compounds under drought conditions may be the result of disruptions in several metabolic pathways in plant cells brought on by stress [47]. Drought stress promotes the formation of reactive oxygen species (ROS) and increases their level in cells combined with changes in carbon uptake, which has an impact on the creation of carbon-based secondary metabolites, in particular phenolic compounds [48]. Furthermore, melatonin can boost the defense systems of plants under abiotic stresses, thus improving the quality of secondary metabolism, which increases the phenolic compounds [49].

3.5. Antioxidant enzymes activity (catalase, and superoxide dismutase) (unit/ g F.W. min)

The activity of both catalase and superoxide dismutase was similar to the secondary metabolites trend as shown in table (4). The highest activity of both catalase and superoxide dismutase (68.69 and 567.20 unit/ g F.W., in order) were noticed in obtained shootlets grown under high intensity of drought stress (2% of PEG) as compared to control that caused the lowest enzyme activity. Likewise, introducing melatonin in the culture medium caused a boost effect on the estimated enzyme activities, where the highest activities of catalase and superoxide dismutase (69.51 and 516.00 unit/ g F.W.) were recorded with added 2g/l of melatonin. Also, these observations were obtained under drought conditions as interacted with melatonin at either 1 or $2gl^{-1}$.

Earlier studies indicated that stress causes several alterations in the antioxidant enzyme activities of plants. These enzymes that are of scavenging ROS include catalase, and superoxide dismutase [50]. In various cycles of physiological processes, adverse external stress induces catalase activity. Catalase detoxifies more ROS when there is stress [51]. Catalase (CAT) can scavenge superoxide after it has been converted to H_2O_2 by superoxide dismutase (SOD), the initial defense enzyme. In this respect, SOD activity has been reported to increase in plants grown under drought [52]. Furthermore, using melatonin under various environmentally harsh conditions such as drought increases the activity of CAT and SOD enzymes [53] [54], as melatonin is an indole molecule generated from tryptophan. It is present in practically all plant species, with amounts varied depending on the plant tissues [55]. It has a considerable impact on plant growth in the face of abiotic challenges with no negative environmental impact [56]. Mayo, et al. [57] mentioned that melatonin regulates the gene expression of antioxidant enzymes in melatonin-treated plants.

4. Conclusions

The results allow us to conclude that MS medium containing 2 gl^{-1} AC, supplemented with 1.0 mg^{-1} of BA + 0.05 mg^{-1} of IBA as well as using melatonin at 2 mg-1 treatment significantly improved micropropagated *K. senegalensis* grown under drought stress conditions, including promoting morphological criteria (number of shootlets, number of roots/shootlet), increasing the content of photosynthetic pigments, secondary metabolite, SOD, and CAT. Melatonin could be a promising natural compound to alleviate impacts of drought stress.This study could be utilized for growers to adapt African mahogany trees to arid environments.

5. Conflicts of interest

There are no conflicts to declare.

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Table 4. Secondary metabolites and activity of enzymes of *in vitro Khaya senegalensis* shootlets grown at different concentrations of melatonin under drought stress**.**

Treatment A	Treatment B	Total phenols (µg/g)	Tannin (µg/g)	Total Flavonoid (µg/g)	Catalase (unit/g F.W.)	Superoxide dismutase (unit/g F.W.)
0% PEG	0 mg L^{-1} Melatonin	16.39 O	6.07 K	3.99 K	41.25 K	269.60 O
	1mg L^{-1} Melatonin	17.55 N	6.87 J	4.20 J	50.00 J	345.70 M
	2 mg L^{-1} Melatonin	18.64 M	6.91 J	4.60 I	50.72 I	371.30 L
0.5% PEG	0 mg L^{-1} Melatonin	19.89 L	7.27 I	4.81 H	50.94 I	307.50 N
	1mg L^{-1} Melatonin	20.23 K	8.68 H	4.92 GH	53.65 H	376.50 K
	2 mg L^{-1} Melatonin	21.08 J	9.06 G	5.17 F	63.76 D	480.00 I
1% PEG	0 mg L^{-1} Melatonin	22.83 I	9.22 FG	4.85 GH	60.31 F	507.30 G
	1mg L^{-1} Melatonin	24.82 G	9.48 EF	4.80 H	61.82 E	500.00 H
	2 mg L^{-1} Melatonin	25.35 F	11.92 D	5.03 FG	69.95 C	551.10 D
1.5% PEG	0 mg L^{-1} Melatonin	23.52 H	9.64 E	5.90 E	51.25 I	411.40 J
	1mg L^{-1} Melatonin	26.11 D	13.06 C	6.63 D	63.48 D	544.10 E
	2 mg L^{-1} Melatonin	25.49 E	13.33 BC	7.45 C	78.09 B	582.3 B
2% PEG	0 mg L^{-1} Melatonin	27.19 C	13.41 B	5.84 E	57.62 G	534.1 F
	1mg L^{-1} Melatonin	30.09 B	12.19 D	7.90 B	63.42 D	572.3 C
	2 mg L^{-1} Melatonin	32.13 A	16.90 A	9.70 A	85.04 A	595.1 A
Mean	0% PEG	17.53 E	6.62 E	4.27 D	47.32 D	328.9 E
	0.5% PEG	20.40 D	8.34 D	4.97 C	56.12 C	388.0 D
	1% PEG	24.33 C	10.21 C	4.89 C	64.03 B	519.5 B
	1.5% PEG	25.04 B	12.01 B	6.66 B	64.28 B	512.6 C
	2% PEG	29.80 A	14.17 A	7.82 A	68.69 A	567.2 A
Mean	0 mg L^{-1} Melatonin	21.97 C	9.12 C	5.08 C	52.27 C	406.0 C
	1mg L^{-1} Melatonin	23.76 B	10.06 B	5.69 B	58.48 B	467.7 B
	2 mg L^{-1} Melatonin	24.54 A	11.63 A	6.39 A	69.51 A	516.0 A

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. PEG: Polyethylene glycol.

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